

Feeding Substrates and Behaviors of Western Cherry Fruit Fly (Diptera: Tephritidae)

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ABSTRACT A study was conducted to determine the abundance of potential foods and the feeding substrates and behaviors of the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), in 2005, 2006, and 2007 in central Washington state. Aphid colonies with honeydew, a presumed food source for flies, were not seen on randomly selected branches of sweet cherry trees, *Prunus avium* L., but leaves with cherry juice, fruit that were damaged, and leaves with bird feces were commonly seen, especially later in the season. Grazing, a behavior in which the mouthparts rapidly move up and down and touch plant surfaces without discrete substances visible to the human eye, was seen more frequently in flies on leaves than on fruit. Grazing occurred more frequently than feeding on extrafloral nectaries (EFNs) on leaf petioles, cherry juice on leaves, and bird feces on leaves. The percentages of females and males that grazed on leaves were not different in 2 of 3 yr, but the percentage of females that grazed was higher in a third year. Percentages of female and male flies that fed on EFNs, cherry juice, and bird feces did not differ. More flies grazed the tops than bottoms of leaves. Flies also grazed on leaves of apple, pear, and grape. The results support the hypotheses that *R. indifferens* feeds mostly on leaves rather than fruit and that leaf surfaces may be the main feeding substrates for *R. indifferens* throughout the season.

KEY WORDS *Rhagoletis indifferens*, feeding ecology, sugar, protein, feeding behavior

An understanding of the feeding ecology and behavior of tephritid fruit fly species can shed light into the evolution of and the phylogenetic basis for feeding strategies among different groups of these economically important insects (Drew and Yuval 2000) and can also have implications for fly management. The foraging behaviors of flies may be shaped in part by the food abundance in the environment (Drew et al. 1983) and within different parts of host plants. From a practical standpoint, understanding the feeding ecology and behavior of flies may help explain how bait sprays work and may also help in the development of feeding attractants, stimulants, or lures for fly management (Lauzon et al. 1998).

Much is understood about the feeding ecology of subtropical or tropical tephritids such as the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Hendrichs and Hendrichs 1990, Hendrichs et al. 1991, Warburg and Yuval 1997a, 1997b), and *Bactrocera* (= *Dacus*) species (Nishida 1958, Drew et al. 1983). In these tephritids, feeding on fruit juices, bird feces, and bacteria takes place on or off host plants, and presumably has evolved to take advantage of their availability in the environment. In particular, nutrition in the form of bacteria may be of overriding importance to the

activity and numbers of fruit flies in the tropics (Drew et al. 1983).

Within the temperate genus *Rhagoletis*, information about feeding ecology and requirements has been derived mostly from studies of the apple maggot, *Rhagoletis pomonella* (Walsh) (Hendrichs and Prokopy 1990, Hendrichs et al. 1993, Prokopy and Papaj 2000), and to a lesser degree the European cherry fruit fly, *Rhagoletis cerasi* L. (Wiesmann 1933), the black cherry fruit fly, *Rhagoletis fausta* (Osten Sacken) (Prokopy 1976), the eastern cherry fruit fly, *Rhagoletis cingulata* (Loew) (Smith 1984), and the western cherry fruit fly, *Rhagoletis indifferens* Curran (Yee 2003a, 2003b). The most comprehensive work on food sources of *R. pomonella* to date suggests this species feeds extensively on leachates (Hendrichs et al. 1993), which are composed of amino acids, vitamins, glucose, fructose, sucrose, bacteria, yeasts, and fungi (Tukey 1971, Lauzon et al. 2003, Lindow and Brandl 2003), and not insect honeydew, which was proposed to be the principal carbohydrate source of *R. pomonella* (Neilson and Wood 1966). Thorough data are lacking for other *Rhagoletis* species, and it cannot be assumed they feed on the same substrates as *R. pomonella*, because host use differs among species.

Rhagoletis indifferens, which is the most important insect pest of sweet cherries (*Prunus avium* L.) in the Pacific Northwest of the United States, is known to

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feed on cherry fruit juice and bird feces in nature (Frick et al. 1954, Yee 2002), but whether these are its principal foods is uncertain. As in other flies, *R. indifferens* require frequent sugar feeding to survive and some protein to develop eggs (Yee 2003a, 2003b), but the foraging behaviors of this fly have not been thoroughly documented. Honeydew has never been shown to be fed on by *R. indifferens* in nature, even though it has been listed as a food for this species (VanRanden and Roitberg 1998, Hammon and Foley 2004). Most trees with high numbers of *R. indifferens* harbor few or no black cherry aphids, *Myzus cerasi* (Fabricius), the major source of honeydew in cherry trees (W.L.Y., unpublished data).

This study was conducted to examine the hypotheses that, throughout the season, (1) *R. indifferens* feeds mostly on undefined matter (to the observer) through grazing on leaf surfaces rather than on fruit and that (2) feeding occurs more frequently on this matter than on visibly defined substances such as honeydew, cherry juice, and bird feces. The grazing type of feeding in *R. pomonella* was referred to as "apparent indiscriminate foraging for diffuse food sources on leaf surfaces of host and nonhost trees" (Hendrichs and Prokopy 1990). Here the behavior is defined as feeding in which the mouthparts rapidly move up and down and touch plant surfaces without discrete substances visible to the human eye. This behavior can be interspersed with brief periods when the mouthparts remain still on the substrate. Because nutritional needs of tephritids such as *R. pomonella* differ by sex (Webster et al. 1979), the hypothesis that (3) there are sex-specific differences in feeding-substrate selection also was tested. Data are compared with published information on other tephritids.

Materials and Methods

Study Sites. The study was conducted in 2005, 2006, and 2007 in central Washington state. Study sites were located in Zillah (46.40° N, 120.26° W, elevation 252 m) and Roslyn (47.22° N, 120.99° W, elevation 695 m). There were four residential yard sites used in Zillah. Site 1 had four sweet cherry trees spaced 3–5 m from adjacent trees and two smaller seedling trees. Site 2 had apple, plum, peach, pear, other fruit trees, and 13 cherry trees. Site 3 had a row of four sweet cherry trees and one sour cherry tree (*Prunus cerasus* L.). Site 4 had five sweet cherry trees. There were three sweet cherry trees used in Roslyn. All trees were 4.5–5 m tall, except the seedling trees, which were ≈2.5 m tall. Trees were not treated with insecticides.

Potential Food Substrates on Trees. In 2005, three randomly selected 1-m lengths of branches 1.5–2 m above ground on each of 12 sweet cherry trees at sites 1, 2, and 3 were tagged (four trees at each site). Leaf and fruit counts on tagged branches were made on 18 May and 17 June. Only leaves originating from the main branch were counted. Numbers of aphid colonies, leaves with cherry juice, numbers of damaged fruit (bruised or opened by birds), and leaves with bird feces on tagged branches were counted on 25

May and 2, 5, 8, 17, and 23 June. Cherry flowers were gone by the time flies emerged, so they were not recorded as a potential food substrate.

Numbers of flies seen on trees over 12 min, including on tagged branches, were also recorded on all 12 trees on 17 and 25 May and 2, 5, 8, 16, 17, and 23 June 2005 from 0900 to 1400 hours (PST) (most flies are seen during these times; Yee 2002) to describe fly population abundance in relation to food abundance. Observations of feeding activities of single flies were not made because the purpose was to survey a high number of trees for foods. No feeding has been observed on tree trunks, so the abundance of potential food sources there was not recorded.

In 2007, extrafloral nectaries (EFNs) of sweet cherry leaves, located on the distal part of leaf petioles, 0–8 mm from the leaves, were sampled for nectar. Collections were made on one tree each at sites 1 and 3 and two trees at site 4. Twenty EFNs from each tree (five EFNs per tree quadrant) were sampled using a 5- μ l microcapillary pipette (VWR International, Leicestershire, UK) once a week on each of three trees for 7 wk from 14 May to 27 June between 0830 and 1100 hours. After the nectar was drawn up, the pipette ends were plugged with wax. Nectar volumes were measured in the laboratory. On 18 May, a sample of nectar was collected from two trees, and its sugar composition was analyzed using an Agilent 1100 Series high-performance liquid chromatography (HPLC) with a refractive index detector (Agilent Technologies, Palo Alto, CA). A 1:4 dilution was made of nectar + solvent (5.0 μ l nectar + 15.0 μ l of a 1:1 acetonitrile:water solvent), and 1 μ l of this mix was injected into the HPLC. Retention times and quantities were compared with known fructose, glucose (Sigma-Aldrich, St. Louis, MO), and sucrose standards (Acros Organics, Geel, Belgium).

Observations of Feeding by Single Flies on Cherry Leaves and Fruit. In 2005, observations of fly feeding were made at site 1 in Zillah on one of the seedling trees and one of the four larger trees on 19, 24, 26, 27, and 31 May and 3, 6, 7, 10, 13, and 14 June between 0830 and 1345 hours. In Roslyn, observations were made on three trees on 5, 7, 11, 14, 15, 18, 20, 25, 27, 29 July and 1, 3, and 4 August between 0900 and 1300 hours. A fly on a leaf or fruit was randomly selected, and its feeding activities were followed for a maximum of 10 min. The first fly that came into view and that could be watched from a distance of 15–25 cm was chosen. Feeding events on leaves or fruit were recorded, and durations of events were recorded with a timer (to calculate percent time grazing). The fly's mouthparts were observed closely. If the fly contacted the substrate with its proboscis, feeding was presumed to occur. Feeding behaviors were recorded as (1) grazing on unidentified matter on leaves or fruit and (2) feeding on discrete substances: (a) nectar from EFNs; (b) cherry juice stains or splatters from damaged fruit; (c) bird feces, and (d) honeydew in or near aphid colonies. Numbers of flies observed depended on fly abundance, but attempts were made to follow at least five females and five males on leaves and fruit each sample

day. A minimum observation of 30 s was required for data to be included in analyses. At Zillah, mean temperatures in the shade (beginning to end of observations) were 19.7–23.8°C and mean relative humidity values were 40–32%, and at Roslyn, they were 22.6–25.1°C and 39–32%, respectively.

In 2006, methods for recording feeding were similar to those in 2005, but there were the following differences: (1) observations were recorded on one tree at site 1 in Zillah (all other trees had been removed by the owner); (2) to determine if flies grazed more frequently on tops versus bottoms of leaves, data of grazing on the two locations were kept separate; (3) to increase the numbers of flies observed, each fly was followed for a maximum of 5 instead of 10 min; (4) all flies were captured using small glass vials after observations to reduce chances of repeated observations of the same fly ($\approx 15\%$ escaped capture); (5) observations were made earlier, usually between 0715 and 1100 hours, because 2005 observations suggested more flies foraged earlier in the day. Flies were observed on 1, 7, 9, 12, 14, and 19 June. On 9 June, observations were made for 1 h from 0600 to 0700 hours. Mean temperatures were 20.8–22.8°C, and mean relative humidity values were 48–42%, except during 0600–0700 hours on 9 June, when temperatures were 14.4–16.9°C and RH values were 55–62%. Observations of fly feeding on EFNs were also made during a study of fly responses to protein baits (data not reported here) on the same tree on 12 dates between 19 May and 9 June. Numbers of observations of feeding on EFNs were recorded and compared with numbers of total sightings of flies during the study.

In 2007, methods for data collecting were similar to those in 2006, with only a few differences. Data of grazing on tops and bottoms of leaves were not kept separate; durations of behaviors were not recorded; and flies at three sites instead of one were observed. Flies were observed on one tree at sites 1 and 3 and on two trees at site 4. Observations were made once a week per tree from 0830 to 1100 hours. There were 21 observation dates: 14, 15, 17, 21, 22, 23, 29, 30, and 31 May and 5, 6, 7, 11, 12, 13, 18, 19, 20, 25, 26, and 27 June. Mean temperatures were 19.8–21.4°C, and mean relative humidity values were 45–34%.

Observations of Feeding Activity on Non-Host Plants. In 2005, at site 2 in Zillah, fly presence or feeding on nonhost plants was recorded on 3, 9, 10, 14, and 15 June. The objective was to determine whether flies are found and/or feed on nonhost plants. Plants observed each day were 3 or 4 pear, 2 or 4 plum, 2 peach, 1 or 2 Asian pear, 3–10 apple, and 1 walnut tree. Non-host trees were 1–3 m from cherry trees. At site 3 in Zillah, observations were made on 14, 17, and 20 June on one grape, one apricot, two plum, and one currant plant. Plants were ≈ 5 m away from cherry trees. Each tree was examined for 5 min, except on 9 and 10 June, when each was examined for 1 min because of time constraints. Fly grazing on leaf surfaces were recorded.

Statistics. For data on potential food substrates on trees in 2005, repeated-measures analysis of variance

(ANOVA) was conducted for each substrate to determine if changes in its abundance occurred over the season. One-way ANOVA was also used to determine differences in mean abundance of each substrate in the months of May and June. Data from six trees were used for May and data from the other six trees were used for June to keep data independent (two trees from each of the three sites for each month). Repeated-measures ANOVA was used to analyze numbers of flies on leaves and fruit on the 12 trees in 2005 and nectar data in 2007. Count data were square-root ($y + 0.5$) transformed. For feeding data, Fisher's exact test (Zar 1999) was used to determine differences in feeding frequencies on leaves versus fruit (2005 data) and in feeding frequencies in May and June in 2005 in Zillah. It was also used to analyze frequencies of flies grazing the leaf versus feeding on other substrates on leaves (EFNs, cherry juice, and bird feces combined); here, using data based on the same samples within a year would violate the assumption of independence of observations, so six comparisons were made: grazing in 2005 versus feeding on other substrates in 2006 and 2007; grazing in 2006 versus feeding on other substrates in 2005 and 2007; and grazing in 2007 versus feeding on other substrates in 2005 and 2006. To compare the percentages of flies feeding on EFNs, cherry juice, and bird feces, a Tukey-type multiple comparison test among proportions (Zar 1999) was used. To ensure data were independent, six analyses were conducted: comparison of EFN in 2005, cherry juice in 2006, and bird feces in 2007; EFN in 2006, cherry juice in 2007, and bird feces in 2005, and so on. Fisher's exact test was used to determine differences in feeding frequencies within substrates between sexes (2005–2007 data). Percent times spent grazing (duration grazing/total time observed) by female versus male flies were subjected to two-way ANOVA (date and sex as factors). Frequencies of grazing on tops versus bottoms of leaves, on both, neither, or of feeding on other substrates were compared using χ^2 . Data were analyzed using SAS (SAS Institute 2004), and means \pm SE are reported.

Results

Potential Food Substrates on Trees. Aphid colonies were not seen on sampled branches of the 12 trees in 2005. However, leaves with cherry juice, fruit that were damaged, and leaves with bird feces were observed (Table 1). Repeated-measures ANOVA indicated a date effect for numbers of leaves with cherry juice, numbers of damaged fruit, and numbers of leaves with bird feces ($F = 34.6, 33.3$, and 18.2 , respectively; all three: $df = 1, 59$; $P < 0.0001$). One-way ANOVA indicated that the abundances in May of all three were lower than in June (Table 1; $F = 23.6$; $df = 1, 10$; $P = 0.0007$, $F = 18.6$; $df = 1, 10$; $P = 0.0015$, and $F = 30.0$; $df = 1, 10$; $P = 0.0003$, respectively). Feeding behaviors of flies in relation to food abundance patterns are described below.

Fly numbers were relatively low in 2005, and no differences in numbers of flies on leaves and fruit were

Table 1. Mean numbers of potential food substrates and populations of *R. indifferens* \pm SE on sweet cherry trees in May and June 2005, Zillah, WA

Date	No. leaves ^a	No. fruit ^a	No. aphid colonies ^{a,b}	No. leaves with juice ^a	No. damaged fruit ^a	No. leaves with feces ^a	No. flies/12-min search		
							Date	On leaves	On fruit
18 May	350.0 \pm 23.7	88.9 \pm 17.8	0	0	0	0.1 \pm 0.1	17 May	3.4 \pm 2.2	0.3 \pm 0.3
25 May	—	—	0	0	0.1 \pm 0.1	1.5 \pm 0.7	25 May	2.8 \pm 1.0	1.7 \pm 0.7
2 June	—	—	0	13.8 \pm 6.3	2.9 \pm 1.1	10.3 \pm 2.6	2–5 June	3.7 \pm 1.2	6.0 \pm 1.9
8 June	—	—	0	7.0 \pm 2.5	0	8.9 \pm 1.5	8 June	3.5 \pm 1.2	2.8 \pm 0.8
17 June	296.3 \pm 20.1	52.9 \pm 11.6	0	14.8 \pm 4.3	5.3 \pm 1.4	4.5 \pm 1.3	16 and 17 June	0.1 \pm 0.1	0.2 \pm 0.1
23–25 June	—	—	0	22.5 \pm 5.4	6.4 \pm 1.6	9.3 \pm 2.7	23 June	0.1 \pm 0.1	0.8 \pm 0.3

^a Means are from the same three 1-m-long branches on each of 12 trees.

^b Potential source of honeydew.

—, not recorded.

detected (Table 1; substrate: $F = 2.2$; $df = 1,129$; $P = 0.1403$; date: $F = 5.3$; $df = 1,129$; $P = 0.0228$; substrate \times date: $F = 2.4$; $df = 1,129$; $P = 0.1280$). The fly population peaked during a period when there were high numbers of leaves with bird feces, before numbers of leaves with cherry juice and numbers of damaged fruit peaked. Bird feces were common, but their sources were unclear. The predominant birds were starlings, *Sturnus vulgaris* L. (83.5% of 820 birds seen on some of the same trees in a 2004 study) and American robins, *Turdus migratorius* L. (9.8%). At least four other bird species were also seen on trees.

The percentages of EFNs sampled in 2007 that had detectable nectar over 7 wk (14 May to 27 June) did not differ (repeated-measures ANOVA, $F = 2.1$; $df = 1,17$; $P = 0.1653$) and averaged $11.2 \pm 3.4\%$ per tree. The volume of nectar/EFN also did not differ over this period ($F = 0.4$; $df = 1,17$; $P = 0.5257$) and averaged $0.020 \pm 0.008 \mu\text{l}/\text{EFN}$ per tree. Sugar analysis detected fructose, glucose, and sucrose in nectar from EFNs. The predominant sugar of the three was sucrose, followed by fructose and glucose, at 73.7, 14.2, and 12.1%, respectively. In addition to *R. indifferens* (below), honey bees, *Apis mellifera* L., and 13 other insect species (bees, wasps, hymenopterous parasitoids, flies, and beetles) were seen feeding on EFNs.

Feeding on Leaves Versus Fruit. In comparisons of grazing on leaves versus fruit in 2005 in Zillah (Table 2),

higher percentages of females ($P = 0.0195$) and males ($P < 0.0001$) grazed on leaves than fruit. Fruit lacked EFNs, and thus this substrate was not compared between leaves and fruit. There were no differences in percentages of female and male flies that fed on cherry juice on leaves versus fruit ($P = 1.000$ and 0.3080 , respectively) and of females that fed on bird feces on leaves versus fruit ($P = 1.000$; no males on bird feces; Table 2). There were more damaged fruit in June than May (Table 1, same pattern at site 1 only), but the percentage of flies (sexes combined) feeding (all forms) on fruit in May (5.5%) was not significantly different than in June (3.6%; $P = 0.6691$).

In Roslyn (Table 2), a difference in grazing on leaves and fruit by females was not detected ($P = 1.000$), but there were only nine females on fruit. A higher percentage of males grazed on leaves than fruit ($P = 0.0067$). There was no difference in the percentages of females that fed on cherry juice on leaves versus fruit ($P = 1.000$).

Grazing Versus Feeding on Other Substrates on Leaves. Within leaves in 2005–2007, higher percentages of flies were seen grazing than performing any other feeding behaviors (Tables 2 and 3). Data from sexes were combined for analyses (see below for justification). Significantly higher percentages of flies were seen grazing on the leaf surfaces than feeding on other substrates in all six comparisons using data from

Table 2. Percentages of *R. indifferens* grazing or feeding on various substrates on sweet cherry leaves and fruit over the season in May to Aug. 2005, Zillah and Roslyn, WA

Behavior/substrate	On leaves		On fruit	
	Females (77)	Males (70)	Females (29)	Males (99)
Zillah				
Grazing	37.7	22.9	13.8	0
EFN	1.3	1.4	—	—
Cherry juice	3.9	4.3	3.4	1.0
Bird feces	1.3	0	0	0
	Females (49)	Males (34)	Females (9)	Males (81)
Roslyn				
Grazing	10.2	11.8	0	0
EFN	0	0	—	—
Cherry juice	4.1	0	0	0
Bird feces	0	0	0	0

Data are from 11 and 13 d of observations in Zillah and Roslyn, respectively.

Numbers of flies observed in parentheses. Each fly observed for max of 10 min. Observations made between 0830 and 1345 hours (PST).

Table 3. Percentages of *R. indifferens* grazing or feeding on various substrates on sweet cherry leaves in June 2006 and May to June 2007, Zillah, WA

Behavior/ substrate	2006		2007	
	Females (131)	Males (130)	Females (216)	Males (164)
Grazing leaf	45.0	45.4	34.3	21.3
EFN	1.5	1.5	1.4	3.7
Cherry juice	6.9	4.6	1.8	0.6
Bird feces	6.1	2.3	1.4	1.2

Numbers of flies observed in parentheses. Each fly observed for a max of 5 min. Except for 9 June 2006, observations were made between 0715 and 1100 hours (PST); 9 June, at 0600–0700 hours.

Data are from 6 and 21 d of observations in 2006 and 2007, respectively.

the 3 yr ($P < 0.0001$, range $P = 4.0 \times 10^{-6}$ to 9.6×10^{-20}).

Grazing on leaves was the predominant feeding behavior in Zillah during May and June 2005, even though a higher percentage of flies was seen grazing in May (39.1%) than in June (16.4%; $P = 0.0052$; sexes combined, no sex difference [$P > 0.05$]). The abundances of cherry juice and bird feces were higher in May than June (Table 1, same pattern at site one only), but there was no significant difference in the percentages of flies feeding on these substrates in May (4.3%) and June (9.1%; $P = 0.2953$).

Feeding on EFNs, Cherry Juice, and Bird Feces. Within other substrates and years, similarly low percentages of flies were seen feeding on EFNs, cherry juice, and bird feces (Tables 2 and 3), although in 2006, relatively high percentages of females fed on cherry juice and bird feces. In six analyses of EFNs, cherry juice, and bird feces across years, only two resulted in significant differences: (1) 2005 EFN, 2006 cherry juice, and 2007 bird feces and (2) 2007 EFN, 2006 cherry juice, and 2005 bird feces ($q_{0.05, \infty, 4} = 3.314$; significant q value = 4.394 and 3.979, respectively). In both cases, the percentage feeding on the cherry juice (5.8%) was greater than on bird feces (1.3 and 0.7%, respectively).

During the 2006 protein bait study, eight females and nine males were seen feeding on nectar of EFNs on 6 of 12 dates. These comprised 4.2% of 188 females and 4.4% of 204 males seen. Females fed on the EFNs for 0.50 ± 0.15 min and males for 0.39 ± 0.09 min ($F =$

0.8; $df = 1,7$; $P = 0.4148$). Feeding occurred at 15.1 – 24.2°C and 36–48% RH.

Feeding on Leaves by Female Versus Male Flies. In 2005 in Zillah, the percentage of females seen grazing on leaves (Table 2) was higher than that of males, but it was not significant ($P = 0.0727$). In 2005 in Roslyn, it was similar in both sexes ($P = 1.000$). In 2006 in Zillah (Table 3), the percentages of females and males that grazed on leaves were similar ($P = 1.000$), but in 2007 in Zillah (Table 3), a higher percentage of females grazed on leaves than males ($P = 0.0061$). In 2005 in Zillah and Roslyn, the percentages of time spent grazing by flies did not differ by sex (Zillah: sex: $F = 1.8$; $df = 1,38$; $P = 0.1913$; date: $F = 0.1$; $df = 2,38$; $P = 0.9195$; sex \times date: $F = 0.01$; $df = 2,38$; $P = 0.9863$; Roslyn: sex: $F = 0.1$; $df = 1,54$; $P = 0.7195$; date: $F = 2.2$; $df = 8,54$; $P = 0.0456$; sex \times date: $F = 1.0$; $df = 8,54$; $P = 0.4691$). This was also true in 2006 in Zillah (sex: $F = 2.6$; $df = 1,103$; $P = 0.1130$; date: $F = 1.2$; $df = 3,103$; $P = 0.3284$; sex \times date: $F = 0.5$; $df = 3,103$; $P = 0.6984$). There were no significant differences in percentages of females and males that fed on EFNs, cherry juice, or bird feces within any year ($P > 0.05$).

Feeding Behaviors on Tops Versus Bottoms of Leaves. In 2006, female and male flies grazed the tops, bottoms, and both sides of leaves during 5-min observations (Table 4), with no detectable differences in percentages of sexes engaged in each behavior ($\chi^2 = 0.25$ – 1.47 ; $P = 0.2253$ – 0.6171). When sexes were combined, a greater percentage of flies first seen on top of leaves grazed on the top than flies first seen on the bottom ($\chi^2 = 40.21$; $P < 0.0001$), but a greater percentage of flies first seen on the bottom grazed the bottom than flies first seen on top ($\chi^2 = 4.55$; $P = 0.0330$; Table 4). A greater percentage of flies first seen on top grazed on both sides of leaves ($\chi^2 = 5.40$; $P = 0.0201$). There was no difference in percentages that grazed on neither ($\chi^2 = 0.64$; $P = 0.4245$), but a greater percentage of flies first seen on top subsequently fed on other substrates ($\chi^2 = 7.36$; $P = 0.0067$; Table 4). There was no difference in grazing durations on top versus bottom of leaves (sexes combined; top, 1.24 ± 0.15 min [$n = 78$]; bottom, 0.99 ± 0.21 min [$n = 22$]; one-way ANOVA, $F = 0.7$; $df = 1,97$; $P = 0.4131$).

Observations of Feeding Activity on Non-host Plants. Over the five dates in 2005 at site 2, 6–48 flies were seen on leaves of all six non-host plant species.

Table 4. Percentages of *R. indifferens* grazing on tops and bottoms of sweet cherry leaves or feeding on other substrates in June 2006, Zillah, WA

	Sex	Total flies	Percent flies subsequently grazing on:				Percent feeding on other substrates
			Top of leaf	Bottom of leaf	Both top and bottom	Neither	
First seen, top	F	80	47.5	2.5	10.0	30.0	10.0
	M	74	39.2	5.4	5.4	47.3	2.7
First seen, bottom	F	49	8.2	14.3	0	75.5	2.0
	M	50	14.0	18.0	6.0	62.0	0

The starting location of eight flies was inadvertently not recorded; these flies not included.

Data are from 6 d of observations.

Grazing on leaves of non-host plants by *R. indifferens* was recorded for the first time. On 9 June, a male fly was seen grazing the leaf of an apple tree. On 14 June, two females were seen grazing the tops of apple leaves and two other females were seen grazing the tops of pear leaves. Over the three dates at site 3, 1–15 flies were seen on leaves of all four nonhost plant species. On 14 June, one female was seen grazing the bottom of a grape leaf.

Discussion

The results of the potential food substrates component of this study indicate that cherry trees had few conspicuous (visible) food substances for *R. indifferens*. Colonies of the aphid *M. cerasi* were not seen in sampled trees, suggesting honeydew is too unreliable a food source for the fly to be dependent on it. Apple and hawthorn trees used by *R. pomonella* also had a paucity of honeydew (Hendrichs and Prokopy 1990). In addition, honeydew was scant in a tropical environment and was not used by *A. fraterculus* (Wiedemann) (Malavasi et al. 1983). Cherry juice and fruit probably are not available food sources for *R. indifferens* during May, because green immature fruit do not produce substantial juice and flies cannot survive on them (Yee 2003b). Bird feces also seem to be too low in abundance in May for flies to benefit from this food during early season. In India, however, there seemed to be benefits of birds and associated feces and fruit damage to tropical fruit fly populations (Grewal and Kapoor 1986). EFNs were abundant, although only a relatively low percentage of them produced nectar throughout the season and mean nectar volume/EFN was low (also see below).

The first hypothesis that feeding through grazing takes place mostly on leaves and not fruit was supported by 2005 data. Leaves appear to dominate the surface area of trees (even when they are loaded with fruit) and are consistently available to flies, unlike suitable fruit. In addition, time spent feeding on fruit, the primary mating site (Yee 2002), may decrease available time for searching for females or guarding mating sites from competing males. In *C. capitata*, males fed on fig fruit late in the day, when they were least likely to find mates (Hendrichs et al. 1991). Similar to *R. indifferens*, percentages of time spent by *R. pomonella* feeding on leaves, fruit, and other substrates were 80, 16, and 4%, respectively (Hendrichs and Prokopy 1990). However, *R. cingulata* reportedly spent little time on leaves and fed as often there as on cherries (Smith 1984). Even though *R. indifferens* rarely fed on fruit, females obtain juice from fruit by stinging them and feeding on the drops that exude from the punctures (Frick et al. 1954). They also feed on damaged fruit (Yee 2002). Feeding on damaged, opened host fruit also occurs in *R. mendax* (Smith and Prokopy 1981), *R. cingulata* (Smith 1984), and *R. turpiniae* Hernández-Ortiz (Aluja et al. 2001), although not in *R. pomonella* (Hendrichs et al. 1993). It is also common in subtropical or tropical tephritids. *A. fraterculus* fed almost exclusively on ripe fruit exuding juice

(Malavasi et al. 1983) and in *C. capitata*, both sexes fed mainly on fruit and not leaves of many host trees (Hendrichs and Hendrichs 1990).

Data from all 3 yr suggest that the main feeding substrate of *R. indifferens* is the cherry leaf and not aphid honeydew, EFNs, cherry juice, or bird feces. This supports the second hypothesis that feeding occurs more frequently on undefined matter on leaves than on visibly defined substances, which is consistent with work on *R. pomonella* (Hendrichs and Prokopy 1990). For *R. indifferens*, perhaps grazing on leaves occurs more often than other feeding behaviors because nutrients are consistently available on leaf surfaces throughout the season. Grazing on leaves was the predominant form of feeding in May and June, even when cherry juice and bird feces abundance increased in June (Table 1). There were also no significant differences in feeding within other substrates between these months, suggesting flies do not depend on them to survive or reproduce.

This study assumes that during grazing, flies were picking up nutrients, which is highly likely (Hendrichs et al. 1993). No attempt was made to discern what foods flies were grazing on, but leachates are a likely possibility (Hendrichs et al. 1993). Leachates apparently are also used by bacteria (Lauzon et al. 2003) that in turn are likely eaten by flies. The leaf surfaces of many woody plants are colonized by bacteria and to a lesser degree by yeasts and fungi (Dickinson 1976, Godfrey 1976, Lindow and Brandl 2003, Lauzon et al. 2003). Bacteria feed on and deplete sugars on the surfaces, but ~20% of sugars still remain on fully colonized leaves (at least of bean) (Mercier and Lindow 2000). There is also evidence that bacteria catabolize leachates, making nutrients more available, and can detoxify allelochemicals (Lauzon et al. 2003). In *Bactrocera tryoni* (Froggatt), diets of bacteria and sugar increased fecundity compared with a yeast and sugar diet (Drew et al. 1983). Despite their availability, nutrients on leaves are spatially heterogeneous (Fiala et al. 1990, Lindow and Brandl 2003) and likely occur in low concentrations, which may explain the extensive grazing by flies on leaf surfaces.

This study recorded for the first time the feeding by *R. indifferens* on EFNs of sweet cherry. Nectar from EFNs contains high concentrations of sugar and a variety of amino acids (more than in floral nectar; in other plants: Baker et al. 1978, Koptur 1994, Tanowitz and Koehler 1986, Wunnachit et al. 1992) and thus seems to be a valuable food. In this study, the predominant sugar detected in cherry nectar was sucrose, which flies can use for survival (Yee 2003b). Feeding on EFNs by *R. indifferens* has not been previously observed (Yee 2002), probably because it occurs infrequently compared with grazing on leaves even though they are abundant, although small (0.8–2.3 mm long, 0.6–1.7 mm wide). The petioles of 100% of 972 mature leaves sampled from the 2006 study tree (in 2005) had EFNs: 20.5, 78.4, and 1.1% had one, two, and three EFNs, respectively (W.L.Y., unpublished data). The relatively low percentage of EFNs with detectable nectar from May through June may be one reason

why flies were rarely seen on them; whether this was caused by other insects removing the nectar is unclear. Frequent feeding on EFNs by *R. indifferens* may never have evolved on bitter cherry, *Prunus emarginata* (Doug. ex Hook.) D. Dietr., the fly's native host, which has EFNs but no detectable nectar using the microcapillary tube method of extraction (W.L.Y., unpublished data). Feeding on EFNs has only been reported for two other *Rhagoletis* species. *Rhagoletis cerasi* fed on EFNs of sweet cherry (Wiesmann 1933) and *R. fausta* fed on EFNs of sour cherry (Prokopy 1976). EFNs were not fed on by *R. cingulata* on its native host, *P. serotina*, which was stated as not having them (Smith 1984), although it does (Tilman 1978). Feeding on EFNs by the subtropical tephritids *Bactrocera cucurbitae* Coquillett and *Bactrocera dorsalis* Hendel has also been recorded (Nishida 1958).

Cherry juice and bird feces seemed to be fed on at similar frequencies by *R. indifferens*, probably because their abundance was similar. Cherry juice may sometimes be fed on more often because at times it can cover a larger surface area than feces. Despite the similar frequencies, the nutrition and effects of these foods differ. Cherry juice has high sugar content, and flies can survive long periods and produce substantial numbers of eggs from feeding on it (Yee 2003b). Bird feces presumably contribute nitrogen (uric acid) and bacteria for egg production, but in the laboratory, they do not increase survival and fecundity in *R. indifferens* (Yee 2003b). This also was true in *R. pomonella* (Hendrichs et al. 1993). Feces may need to be combined with other substances in nature to benefit egg development. In *C. capitata*, feces added to a diet of figs increased fecundity (Hendrichs et al. 1991). Bird diet may also affect the nutritional quality of feces to flies. Bird feces are fed on by *R. fausta* (Prokopy 1976), *R. cingulata* (Smith 1984), *R. pomonella* (Hendrichs and Prokopy 1990), and *C. capitata* (Hendrichs et al. 1991, Warburg and Yuval 1997a). However, even when there was a high abundance of bird feces, relatively little feeding on them by *C. capitata* was observed (Warburg and Yuval 1997a).

The third hypothesis that there are sex-specific differences in feeding-substrate selection was not supported by most comparisons in this study. Grazing on leaves by both sexes was similar perhaps because food locations on cherry trees are limited, so there are few options to drive the evolution of different feeding strategies between sexes. However, in 2007, more females than males were seen grazing, suggesting that under some unknown environmental conditions, females need more nutrients than males. Feeding by both sexes on leaves, EFNs, and cherry juice may be similar because both are highly dependent on sugars. Similar feeding patterns by females and males on bird feces is difficult to explain, because in male *R. pomonella*, nitrogen or protein may not be needed for spermatogenesis (Webster et al. 1979). However, feeding by male *R. indifferens* on bird feces suggests males, like females, need more nitrogen.

Grazing by female and male flies occurred more frequently on tops than bottoms of cherry leaves,

suggesting more food occurs on the tops than bottoms. Flies foraging on tops of leaves may more likely find cherry juice and bird feces. In *R. cingulata*, all feeding while on leaves occurred on the upper surface of the leaf blade (Smith 1984). *R. indifferens* seems to use the bottoms of cherry leaves more for resting, shelter, and protection from sun or natural enemies than for feeding.

Grazing on non-host leaves, specifically of apple, pear, and grape, by *R. indifferens* was observed for the first time. Feeding on nonhost plants was seen about one third of the time in *R. fausta* (Prokopy 1976) and also occurs in *R. cerasi* (Katsoyannos et al. 1986) and *R. pomonella* (Hendrichs and Prokopy 1990). This supports the notion that *Rhagoletis* flies are opportunistic feeders (Prokopy and Papaj 2000). The occurrence of *R. indifferens* on six non-host plants adjacent to cherry trees suggests the opportunities to feed on these plants are high.

The data in this study have implications for use of baits for management of *R. indifferens*. Insecticide or bait droplets on leaves, especially on their tops, are highly likely to be found and eaten by flies through chance encounters while grazing on leaves. However, adding attractants in baits may quicken response times by the flies. Whether EFNs, cherry juice, or bird feces can compete with baits is unknown, but in *R. pomonella*, bird feces may compete with them (Prokopy et al. 1993).

The results of this study provide new insights into the feeding ecology of *R. indifferens*, but further work is needed to fully understand the fly's nutritional ecology and requirements. Specifically, the identities and quantities of materials present on cherry leaves and on fruit and in EFNs, cherry juice, and bird feces need to be determined. Amounts of materials ingested from all substrates also need to be determined. Determining these will help us better understand how the feeding behaviors of *R. indifferens* evolved and why they may differ from those of other *Rhagoletis* and tephritid species. From a practical standpoint, this may help in the development of attractants or feeding stimulants in lures or baits for fly management.

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